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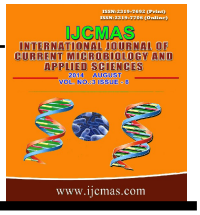

Original Research Article

Isolation of Intestinal Microflora and its Probiotic Effect on Feed Utilization and Growth of Gold Fish *Carassius auratus*

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Keywords

Isolation, intestinal microflora, probiotic effect, feed utilization, growth, Gold fish

A B S T R A C T

Two distinct colonies were isolated from the intestine of Gold fish *Carassius auratus*. Based on biochemical tests, the isolated colonies were identified as Pseudomonas and Proteus spp. Among the identified microbes mass multiplication of Pseudomonas in Nutrient broth was carried out. Five different feeds having different concentration of microbes such as Feed I (Control), Feed II(10^5 cells of pseudomonas spp), Feed III(10^6 cells), Feed IV(10^7 cells) and Feed V(10^8 cells) were prepared by using fish meal, groundnut oil cake, wheat flour and tapioca flour. Feed utilization parameters were studied after a period of 45 days. The feed conversion efficiency, growth, percentage growth, relative growth rate, metabolism and net growth efficiency were higher in feed I. The feed consumption, feed conversion ration and gross growth efficiency were higher in feed V.

Introduction

In ornamental fish culture disease outbreaks are being increasingly recognized as a significant constraint on production and trade, affecting the economic development of many countries. So far, conventional approaches such as the use of disinfectants and antimicrobial drugs have had limited success in the prevention or cure of aquatic diseases. One of the most significant technologies that have evolved in response to disease control problems is the use of probiotics[Browdy, 1998].Probiotics are live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance and that compete the harmful one and thus protect the host. The microflora present in the intestinal tract generally seems to be from the environment or diet which can survive and multiply in the intestinal tract. The work related to the isolation of intestinal micro flora and its probiotic effect on feed utilization and growth of Gold fish Carassius auratus is totally wanting although few studies are available in food fishes and other organisms[Yashimizu et al 1976., Montes and Pugh 1993., Douillet and Langdon.1994 and Ashraf Ali.2000]. Hence the present study was carried out.

Materials and Methods

For the present study gold fish (2.30± 0.45 g) were collected from Angel Aquarium, Dindigul, Tamil Nadu, India and transported to the laboratory in polythene bags filled with aerated water. Intestinal contents from the gold fish were serially diluted with sterile water and plated on nutrient agar. Plates were incubated at 37º C for 24 hours. After incubation bacterial colonies were invalid at random from each plate, and examined for gram reaction, spore formation, cellular morphology, motility and identified at the genus level. Based on biochemical test, the isolated colonies were identified as pseudomonas and proteus spp.

Mass culture of isolated bacteria:

The isolated pseudomonas culture was mass multiplied by inoculating them in to the nutrient broth. After that the optical density has been determined for each dilution of the mass multiplied culture at spectrophotometer. The ratio of cells from 10^5,10^6,10^7 &10^8 was mixed along with the feed. Determined the cell count(CFU) against optical density using semilogarithmic graph.

Experimental Feed Preparation:

Ingredients used in the feed were fish meal, groundnut oil cake, wheat flour, tapioca, fish oil, sunflower oil, supplevite – mix, sodium chloride and sodium benzoate. After knowing the protein content of major ingredients by Micro –kjeldhal method [Jeyaraman, 1992] the feed were prepared according to square method [Ali, 1980]. The components used for feed preparation was dried, powdered and sieved through 425 microne sieve.The major ingredients were weighed and mixed thoroughly with 130 – 150 ml of distilled water. The mixed stuff was put in autoclave for 15 min. at 100º C and cooled. After cooling, cultured pseudomonas species in the different cells such as 10^5,10^6,10^7 &10^8 are mixed with the feed, then it was extruded with the help of pelletizer. The pellets were dried in room temperature. The formulated feed was kept in air tight container at -20º C until used to prevent contamination (Table 1).

Experimental design for growth studies:

For growth studies Gold fish(2.30 ± 0.45 g) were collected and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in plastic round aquaria60 cm dia.) for 15 days. After 15 days uniform size of Gold fish were selected and weighed. Five fishes were introduced in rectangular glass tanks(45 cm L X 22 cm B) having a capacity of 20 liters. During rearing, fishes were fed on ad – libitum diet on prepared feed twice a day for 1 hour each from 8-9am and 4-5 pm. The unfed were collected after one hour of feeding without disturbing the fishes. The unfed was dried to constant weight. The faecal matter was collected daily before changing the water with least disturbance to the fishes and dried at 65º C. Approximately 70% of water in the glass tank was replaced with fresh water. The experiment was continued for 45 days and on the 46^{th} day the fishes were weighed in live condition. Feed utilization parameters were calculated.

Results and Discussion

The experimental work indicates that the isolated bacteria is identified as Pseudomonas spp. The fresh water fish harbour human pathogenic bacteria including Aeromonas spp, Enterococcus facials, Escherichia coli, Salmonella spp and Staphylococcus spp. In their intestine [Geldrich and Clarke, 1996].

Table.1 Composition of different ingredients in Experimental feeds (g / 100g)

S.No.	Ingredients	Experimental Feeds				
		I	II	III	IV	V
1.	Fish meal	34.15	34.15	34.15	34.15	34.15
2.	Groundnut oil cake	34.15	34.15	34.15	34.15	34.15
3.	Wheat flour	10.85	10.85	10.85	10.85	10.85
4.	Tapioca	10.85	10.85	10.85	10.85	10.85
5.	Fish oil	2	2	2	2	2
6.	Sunflower oil	2	2	2	2	2
7.	Supplevite – mix	4	4	4	4	4
8.	Sodium chloride	1	1	1	1	1
9.	Sodium benzoate	1	1	1	1	1
10.	Pseudomonas (cells)	-	10^5	10^6	10^7	10^8

Table.2 Feed utilization and Growth of Gold fish *Carassius auratus* in relation to different cells of Pseudomonas spp. Each value is the average (± S.D) performance of 5 individuals in triplicate reared for 45 days

S.No.	Parameters	Feed I (Control)	Feed II (10^5 cells)	Feed III (10^6 cells)	Feed IV (10^7 cells)	Feed V (10^8 cells)
1.	Feed Consumption(FC) (g / g live wt/ 45 days)	1.29 ± 0.04^a	1.35 ± 0.10^b	1.43 ± 0.21^c	1.39 ± 0.16^d	1.54 ± 0.03^e
2.	Feed Conversion Efficiency(FCE)	0.22 ± 0.03	0.13±0.03	0.17±0.05	0.16±0.07	0.11±0.03
3.	Feed Conversion Ratio (FCR)	0.94 ± 0.08	1.66±0.41	1.27±0.36	1.43±0.73	1.95±0.63
4.	Growth (G) (g / g live wt / 45 days)	1.4 ± 0.17^a	0.83 ± 0.09^b	1.16 ± 0.21^c	1.1 ± 0.4^d	0.76 ± 0.32^e
5.	Percentage Growth (PG) (%)	66.98 ±10.9	38.93±10.0	51.88±15.7	51.79 ±14.5	31.51±10.12
6.	Relative Growth Rate (RGR)	1.3 ± 0.08	0.38±0.02	0.55±0.13	0.55±0.2	0.38±0.16
7.	Assimilation (A)	0.87 ±0.02	1.07±0.07	1.12± 0.23	1.06±0.15	1.21±0.08
8.	Metabolism (M)	0.52 ±0.16	0.26±0.19	0.31±0.20	0.40±0.13	0.45±0.19
9.	Gross Growth Efficiency (GGE) (%)	11.23 ± 1.28^a	6.27 ± 1.67^b	8.41 ± 2.85^c	8.13 ± 2.5^d	12.47 ± 10.7^e
10.	Net Growth Efficiency (NGE) (%)	17.0 ± 0.36^a	7.86 ± 2.00^b	1.82 ± 1.09^c	10.78 ± 2.8^d	6.45 ± 2.9^e

Feed Consumption	Growth	Gross Growth Efficiency	Net Growth Efficiency
aVs b ($p > 0.05$) S	aVs b ($p > 0.05$) NS	aVs b ($p > 0.05$) NS	aVs b ($p > 0.05$) S
aVs c ($p > 0.05$) S	aVs c ($p > 0.05$) NS	aVs c ($p > 0.05$) NS	aVs c ($p > 0.05$) S
aVs d ($p > 0.05$) S	aVs d ($p > 0.05$) NS	aVs d ($p > 0.05$) NS	aVs d ($p > 0.05$) S
aVs e ($p > 0.05$) S	aVs e ($p > 0.05$) NS	aVs e ($p > 0.05$) S	aVs e ($p > 0.05$) S

S – Significant NS – Non - Significant

Many workers have isolated bacteria from marine sources, fish with probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod [Guildberg, et al., 1995].

The feed utilization and growth parameters of Gold fish were presented in Table 2. Feed consumption of gold fish was higher in feed V containing 108 cells of Pseudomonas spp. In feed I the feed consumption was lower. The feed consumption significantly varied in different feeds. Feed conversion efficiency was higher in feed I. Feed conversion ratio was best (1.95) in feed V. Growth of gold fish were higher in feed I and lower in feed VF. Like growth, the percentage growth and relative growth rate was higher in feed I. Assimilation and metabolism was higher in feed V and I respectively. The gross growth efficiency and net growth efficiency was higher in feed V and I respectively. Browdy[1998] demonstrated that the probiotic effect of bacterial mixture of feed to fishes will lead to great growth and high survival rate. Several authors studied the isolation of intestinal bacteria and its effect on growth and survival of different fishes and other organisms [Guildberg et al 1995., Ringo and Gatessoupe,1997., Harzevili et al1998 and Rengpipat et al,1998].

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